Appl. No. 10/531,475

Atty. Ref.: 5547-2

Amendment After Final Rejection

March 23, 2010

## **REMARKS**

Reconsideration is requested.

Claims 1, 4, 10, 13, 14, 40, 43, 44, 47 and 48 are pending.

The claims have been revised, without prejudice. The applicants submit that the claim revisions find support throughout the specification and that no new matter has been added.

The Section 112, first paragraph "enablement", rejection of claims 1, 4, 10, 13, 40, 43, 44 and 47 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following comments.

Claims 1, 4, 10, 13, 40, 43, 44 and 47 have been rejected as allegedly not being supported by an enabling disclosure. According to the Examiner, the effect of introduction and expression of a specific nucleic acid in a plant cell would be unpredictable. Specifically, the Examiner refers again to the documents Sakamoto (Plant Physiol. 2004 Sep; 136(1):2734-46) and Temple (Plant Mol Biol. 1998 Jun; 37(3):535-47).

According to the Examiner, Temple discloses the introduction of antisense constructs targeting two glutamine synthetase 1 genes (GS-1) in alfalfa. They observe a reduction at the transcript level of about 80%. However, no reduction in glutamine synthetase activity was observed. Therefore, the Examiner is believed to have incorrectly concluded that the introduction of a gene does not necessarily have an effect.

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According to the present invention, the polynucleotide having a sequence as set forth in SEQ ID NO:1835 shall be (over-)expressed, rather than down-regulated. Overexpression and down-regulation, however, are two completely different approaches. The constructs introduced by Temple are antisense constructs directed against two members of a gene family (GS-1). As previously explained, there may be more than two glutamine synthetases 1 in alfalfa. Presumably, the existence of more than 2 GS-1s would explain why Temple continued to observe GS-1 activity. The applicants believe that one of ordinary skill in the art will find it likely that Temple simply did not target all GS-1s.

The genome of alfalfa, however, is not completely sequenced. The applicants have searched for the number of GS-1s in Arabidopsis whose genome has been completely sequenced. According the TAIR website (www.arabidopsis.org) there are believed to be at least 3 GS-1s. The applicants note that the genome of Arabidopsis is much smaller than the genome of Alfalfa, i.e., Alfalfa may comprise even more than 3 GS-1s. Therefore, it is likely, that the remaining enzyme activity observed by Temple is caused by the expression of further GS-1s whose expression was not down-regulated.

According to the Examiner, Sakamoto would have introduced a nucleic acid encoding for AZF2 (i.e., for SEQ ID NO:1835) under control of the 35S promoter in Arabidopsis. However, Arabidopsis plants over-expressing SEQ ID NO:1835 could not be obtained. Therefore, the Examiner concludes that plants transgenic for a sequence that is natively expressed are not always predictably obtainable.

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Experimental results have been previously presented in which a nucleic acid encoding for SEQ ID NO:1835 was operably linked to either a constitutive promoter (GOS2), or a seed-specific promoter (prolamin promoter) was introduced into plants. The applicants note that Sakamoto used the CaMV35S promoter, which is a constitutive promoter. The resulting plants showed increased yield.

The applicants note for completeness that the polynucleotide that was linked to the GOS2 and the prolamin promoter in the experiments described previously was not exactly a polynucleotide having a sequence as shown in SEQ ID NO:1835, since the protein encoded by said polynucleotide differed in one amino acid from a protein encoded by SEQ ID NO:1835. The expressed protein showed 99.6% sequence identity to the protein encoded by SEQ ID NO:1835 (i.e. to a protein having a sequence as shown in SEQ ID NO:1836).

Withdrawal of the Section 112, first paragraph "enablement", rejection is requested.

The Section 112, second paragraph, rejection of claim 1 is obviated by the above amendments. Entry of the present Amendment will at least reduce this issue for appeal. Entry of the present Amendment and withdrawal of the rejection are requested.

The Section 102 rejection of claims 14 and 48 over De Veylder (EMBO, Journal 2002, March 15; 21(6):1360-8), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following distinguishing comments.

The Examiner is understood to believe that the plants taught by de Veylder inherently comprise a nucleic acid sequence which is at least 95% identical to SEQ ID

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NO:1835 (claim 14) or which is at least 95% identical to a sequence encoding SEQ ID

NO: 1836 (claim 48).

Claim 14 however describes a transgenic plant comprising an isolated nucleic acid sequence which is at least 95% identical to SEQ ID NO:1835. The polynucleotide shown in SEQ ID NO: 1835 is a cDNA. In contrast, the wild-type plants taught by de Veylder comprise the genomic sequence encoding for AZF2, and, thus, a sequence which additionally comprises regions encoding for introns. Accordingly, de Veylder does not disclose plants comprising an isolated nucleic acid sequence which is at least 95% identical to SEQ ID NO:1835. Thus, claim 14 is not anticipated by the cited art.

Moreover, claim 48 above describes a transgenic plant comprising a heterologous nucleic acid sequence which is at least 95% identical to a sequence encoding SEQ ID NO:1836. The nucleic acid identified by de Veylder et al as being down-regulated in Arabidopsis is homologously expressed in Arabidopsis. Accordingly, the cited art fails to anticipate the invention of claim 48.

It is further noted that claims 14 and 48 could not have been obvious in light of the disclosure of de Veylder as there is no over expression of a nucleic acid as shown in SEQ ID NO:1835 in plants. Rather, de Veylder observed reduced expression of SEQ ID NO:1835 in plants expressing certain transcriptions factors.

Withdrawal of the Section 102 rejection is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned in the event anything further is required.

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Respectfully submitted,

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Your query for genes where gene name, description, phenotype, locus name, uniprot id or GenBank accession contains the term glutamine synthetase 1 resulted in 3 tool matches with 3 distinct gene models.

Displaying 1 - 3.

To see ESTs associated with your gene of interest, click on the Locus link.

	Locus	Description	Gene Model(s) Ø	Other Names	Keywords 🚱
	AT1G48470	Encodes cytosotic glutamine synthase isozyme. Expression of mRNA is not detectable in roots.	AT1G48470.1	GLN1 5 GLUTAMINE SYNTHETASE 1 5 T1N15.8 T1N15_8	4 anthesis, E expanded cotyledon stage, L mature polion stage, carpel, chloroptast, flower, giutamate-ammonia ligase activity, giutamine biosynthetic process, leaf, leaf whori, male gametophyte, nitrogen compound metabolic process, petal, petal differentiation are expansion stage, seed, sepal, shoot apex, stamen
		encodes a cytosolic glutamine synthetase, the enzyme has low affinity with substrate ammonium	AT3G17820.1	ARABIDOPSIS THALIANA GLUTAMINE SYNTHASE CLONE KB6 ATGSKB6 GLN1.3 GLN1 3 GLUTAMINE SYNTHETASE 1.3 GLUTAMINE SYNTHETASE 1.3 GLUTAMINE SYNTHETASE 1 3 MEB5.4	4 anthesis, 4 leaf senescence stage, C globular stage, D bilateral stage, E expanded cotyledon stage, F mature ambryo stage, L matupollen stage, LP.02 two leaves visible, LP.04 four leaves visible, LP.06 six leaves visible, LP.08 eight leaves visible, LP.10 ten leave visible, LP.12 twelve leaves visible, M germinated pollen stage, carpel, cauline leaf, chloroplast, copper ion binding, cotyledon, cultured cell, cytosol, cytosolic ribosome, embryo, flower, glutamate ammonia ligase activity, glutamine biosynthetic process, hypocotyl, inflorescence maristem, leaf, leaf apex, leaf lamina base, leaf whort, male gametophyte, nitrate assimilation, pedicel, petal, petal differentiation and expansion stage, petiole, plasma membrane, politube, response to cadmium ion, root, seed, seedling growth, sepal, shoot, shoot apex, stamen, stem
T3		Encodes a cytosolic glutamine synthetase, the enzyme has high affinity with substrate armonium	AT6G18570.1	GLN1 4 GLUTAMINE SYNTHETASE 1 4 MTG13.1	4 anthesis, 4 leaf senescence stage, E expanded cotyledon stage, LP.02 two leaves visible, LP.04 four leaves visible, LP.05 six leaves visible, LP.08 eight leaves visible, LP.10 ten leaves visible, LP.12 twelve teaves visible, carpet, cauline leaf, cytosol, embryo, flower, glutamate-animonia ligase activity, inflorescence meristem, leaf, leaf lanina base, leaf whork, nitrate assimilation, petal, petal differentiation expansion stage, petiole, root, seed, sepal, stamen, stem

Gene Search Results

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General comments or questions: curetor@arabidopsis.org Seed or DNA stock questions (donations, availability, orders, etc): abro@arabidopsis.org

